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Evidence for a Physiological Role of Gonadotropin-Releasing Hormone (GnRH) or GnRH-Like Material in the Ovary*

LUTZ BIRNBAUMER, NAHID SHAHABI, JEAN RIVIER, AND WYLIE VALE

Department of Cell Biology, Baylor College of Medicine (L.B., N.S.), Houston, Texas 77030; and the Peptide Biology Laboratory, Salk Institutes (J.R., W.V.), San Diego, California 92138

ABSTRACT. The possibility that GnRH or a GnRH-like material of ovarian origin may play a physiological role in follicular development was explored in immature hypophysectomized rats by testing whether a potent synthetic antagonist of GnRH action ([*N*-acetyl-dehydro-Pro^1,D-*p*-chloro-Phe^2,D-Trp^{3,6}]GnRH), would potentiate FSH-induced maturation of ovarian follicles to an ovulable stage. Rats were hypophysectomized on day 25 of their life and implanted with a Silastic capsule containing diethylstilbestrol. On day 30, they were started on injections of 10 μg NIH FSH-S12 twice daily alone (control) or in combination with 10 μg of either native GnRH or GnRH antagonist. On day 35, all rats received 30 IU hCG to trigger ovulation and luteinization of mature follicles. Rats were killed 25.5–28 h later and inspected for number of ova in Fallopian tubes, ovarian weight, number of corpora lutea (CL) on ovarian surface, and appearance of hematoxylin-eosin-stained ovarian slices. In control animals (n = 6), we found some ovulations (mean ± SEM, 3.2 ± 1.1/rat), many more CL (16.5 ± 4.5/rat), and ovarian weights of 37.7 ± 1.1 mg/rat. In GnRH-treated rats (n = 5), there were no CL formed, no ova were found, and ovarian weights were 16.0 ± 1.5 mg/rat. In contrast, in GnRH antagonist-treated rats (n = 5), 16.4 ± 1.6 ova/rat were recovered from the Fallopian tubes, and ovaries contained 20.8 ± 2.5 CL/rat and weighed 52.7 ± 3.2 mg/rat. All changes were statistically significant. We conclude that an antagonist of GnRH action is able to potentiate the action of FSH on ovarian follicle development and suggest that it does so by inhibiting the action of an endogenous GnRH or GnRH-like substance that may play a role as a physiological atretic signal. (*Endocrinology* **116:** 1367–1370, 1985)

THERE is ample evidence that GnRH affects ovarian functions in the rat. Receptors for GnRH have been characterized in ovarian granulosa and luteal cells and administration of GnRH to hypophysectomized rats has been shown to result in a variety of both inhibitory and stimulatory responses, including follicular and luteal regression and ovulation (for reviews, see Refs. 1 and 2, also 3–6). In spite of these well documented direct actions on the ovary, there is no clear evidence for GnRH formation in the ovary or for a physiological role of the decapeptide. The possibility exists, therefore, that the effects of GnRH are of a pharmacological nature. Based on the fact that the specificity and affinity properties of ovarian GnRH receptors are essentially indistinguishable from those of pituitary GnRH receptors (7), it stands to reason that an ovarian GnRH-like peptide should exist and that it should play a physiological role. The work presented below was designed to examine whether a GnRH-like material is acting in the ovary by testing for effects of a very potent synthetic GnRH antagonist (8) on follicular development and maturation. Specifically, we made use of the well established observation that both *in vivo* (9) and *in vitro* (10, 11) GnRH inhibits the follicle-maturing effects of FSH and tested whether the administration of GnRH antagonist to hypophysectomized and estrogen-treated rats would lead to altered FSH-induced maturation of follicles to an ovulable stage. The results show that administration of GnRH antagonist together with FSH leads to a significant increase in the number of follicles susceptible to ovulation upon hCG administration. They strongly suggest that GnRH or GnRH-like substances are present and active under normal physiological conditions.

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Address requests for reprints to: Dr. Lutz Birnbaumer, Department of Cell Biology, Baylor College of Medicine, Texas Medical Center, Houston, Texas 77030.
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Materials and Methods

Animals and sources of hormones

Female hypophysectomized rats, 25 days of age, were purchased from Hormone Assay Laboratories (Chicago, IL). GnRH was purchased from Penninsula Laboratories (San Carlos, CA). hCG (~1000 IU/mg) and diethylstilbestrol (DES) were obtained from Sigma Chemical Co. (St. Louis, MO). FSH (NIH S12) was supplied to us through courtesy of the Hormone Distribution Officer (NIH). GnRH antagonist ([*N*-acetyl-dehydro-Pro^1,D-*p*-chloro-Phe^2,D-Trp^{3,6}]GnRH) was synthesized as described previously (12).

Treatments

Female immature rats were hypophysectomized on day 25 by the transaural method by Hormone Assay Laboratories. On the same day, they received a 10-mm Silastic implant containing DES, prepared exactly as described by Louvet *et al.* (13). The animals were subsequently shipped to Houston and housed in animal quarters with a 0600 h lights-on, 0600 h lights-off lighting schedule. At 0800 h on day 30, the rats were subdivided into three groups. Group I received five sc injections at 12-h intervals, each of 10 μg FSH in 0.4 ml saline (control). Group II received the same treatment, except that with each injection of FSH they also received 10 μg native GnRH. Group III was treated as group II, except that 10 μg GnRH antagonist replaced GnRH. At 1430 h on day 32, each rat received 30 IU hCG to promote ovulation and luteinization of those follicles that had matured to that stage. The rats were then killed between 1600–1830 h on the next afternoon (day 33) and examined for 1) number of ovulated ova present in the Fallopian tubes, 2) corpora lutea (CL) formed on the surface of the ovaries, 3) ovarian weights, and 4) microscopic appearance of the ovarian structures (14). This treatment protocol is summarized in Fig. 1.

All other procedures and materials have been described previously (14).

Results

In 1975, Louvet *et al.* (13) reported the basic protocol employed here for eliciting maturation of ovarian follicles in hypophysectomized immature rats. We used ovulation as the end point for testing for effects of FSH on follicular maturation because this is probably the most stringent way to evaluate the completeness of the action of FSH in the otherwise abnormal animals. By obtaining ovulation, we felt that we were mimicking, without the use of LH (15), the physiological milieu that leads to formation of mature follicles as closely as possible. We found that by following the protocol of Louvet *et al.* (13) using a submaximal dose of DES (Silastic capsule of 10 mm), we were able to obtain not only development of antral follicles but also some ovulations. Further, initial trials showed that coadministration of FSH and GnRH antagonist resulted in visibly larger ovaries and an enhanced number of ova recovered from the Fallopian tubes. O the basis of this, the experiment schematized in Fig. was set up.

Figure 2 presents light micrographs of representative ovaries from rats treated with FSH alone (control), FSH plus GnRH (GnRH), and FSH plus GnRH antagonist (GnRH antagonist) all at a magnification of ×7. Table 1 summarizes the effects of the treatments on ovarian weights, number of ova recovered from the Fallopian tubes, and number of CL formed. It can be seen that, as expected on the basis of work by others (9–11), GnRH administration resulted in a complete inhibition of development of ovulable follicles, as seen by the lower weights of the ovaries, lack of development of antra, and lack of formation of luteinizing structures. In addition (*inset to first panel* of Fig. 2), many oocytes were found that had undergone cellular division (16).

In contrast to the results with GnRH, coadministration of FSH and GnRH antagonist resulted in a significant increase over control in the number of ova recovered from the Fallopian tubes, *i.e.* in the number of follicles that had matured to an ovulable stage under the conditions of the experiment (Table 1). In agreement with the increase in number of ovulations elicited, rats that had received FSH in combination with GnRH antagonist also had an increased number of newly formed CL, and their ovaries were heavier than those of their counte parts, with a lower number of new CL in control rats. On the basis of examinations of hematoxylin-eosin-stained slices (5 μg thick) under the light microscope, such as shown in Fig. 2, GnRH antagonist treatment did not seem to have any detectable effect on cellular structure or follicular appearance when cells or follicles were considered as individual entities. The effect of the GnRH antagonist appears to have been merely one of increasing the number of successfully recruited follicles. It should be pointed out, however, that this observation is based purely on observing hematoxylin-eosin-stained tissue sections, and more detailed investigations into the ultrastructural properties of the various cell types involved (thecal cells, granulosa cells, and oocytes) and their

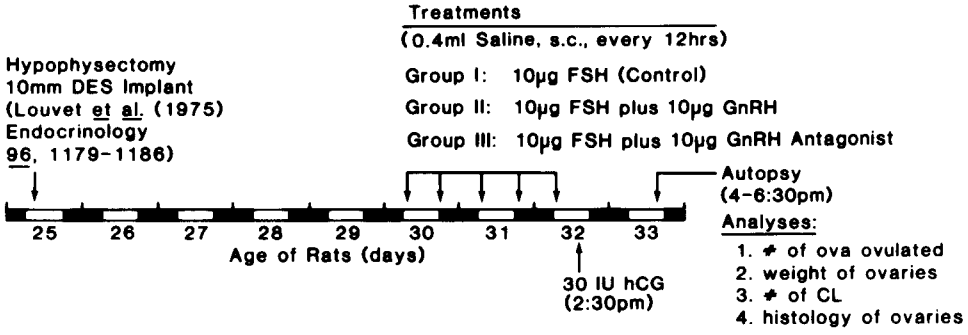

FIG. 1. Summary of treatment schedule and measurements made.

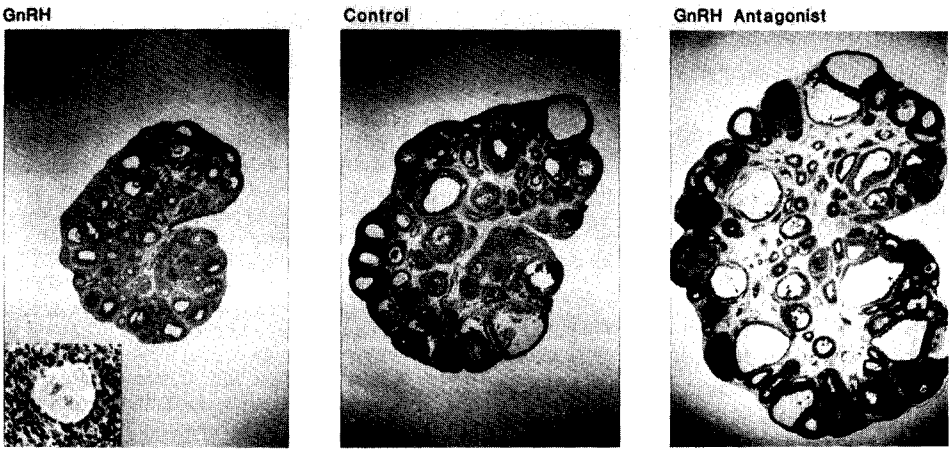

FIG. 2. Photomicrographs of ovaries from immature, hypophysectomized, and DES-implanted rats that had received for 2.5 days FSH alone (control), FSH plus GnRH (GnRH), or FSH plus GnRH antagonist (GnRH antagonist), sampled 25 h after hCG injection. Magnification, ×7. *Inset,* Higher magnification showing divided oocyte in ovary of FSH plus GnRH-treated rat.

TABLE 1. Effect of treatments on ovulation-related parameters

	Treatment		
	Control	GnRH antagonist	GnRH
Ovarian wt (mg/rat)	37.7 ± 1.1 (6)	52.7 ± 3.2 (5)	16.0 ± 1.5 (5)
No. of ova found in Fallopian tubes			
Left	1.2 ± 0.4 (5)[a]	16.4 ± 1.6 (5)	None
Right	2.2 ± 1.0 (6)	12.2 ± 2.9 (5)	None
Total/rat	3.2 ± 1.1 (6)	28.6 ± 4.5 (5)	None
No. of CL present on ovarian surface			
Left	6.2 ± 1.3 (5)[a]	20.8 ± 2.5 (5)	None
Right	9.8 ± 2.9 (6)	16.8 ± 3.3 (5)	None
Total/rat	16.5 ± 4.5 (6)	37.6 ± 3.0 (5)	None

Values are the mean ± SE.

[a] The left ovary of one of the rats was undeveloped and/or atrophied.

biochemical properties, such as LH receptors, aromatase levels, and prostaglandin production, may show differently.

A discrepancy was found between the number of ova that could be recovered from the Fallopian tubes and the number of ova expected on the basis of the number of clearly luteinizing structures that were discerned on the surface of the ovaries. This was especially marked in the control group, where an average of 3.2 ova were found per rat, while as many as 16.5 CL existed. Thus, only 20% of as many ovulated ova were found as expected from the CL present. By examining serially sectioned ovaries from the rats of the control group, we discovered that 38% of the luteinized structures still contained an oocyte. In the FSH plus GnRH antagonist-treated group, 76% of the theoretically expected ovulated ova were found (Table 1). By examining the number of the CLs in this group that still contained oocytes, we found that only 16% of them had not ovulated. Thus, while in the control group only 59% of the oocytes could be accounted for, in the FSH plus GnRH antagonist-treated group it was possible to account for up to 92% of the oocytes of those follicles that luteinized. The reason(s) why we could not account for all of the oocytes in this group is not clear. All nonovulated follicles were found to contain oocytes. It seems reasonable to assume, therefore, that follicles that had been induced to luteinize also contained an oocyte at the time of hCG administration. We have not investigated further the reason we were unable to find close to 40% of the ovulated ova in control rats.

Discussion

GnRH has been firmly established as blocking FSH effects on preantral follicles both *in vivo* and *in vitro* (9–11). Since the overall role of FSH is to promote maturation (development of the preantral follicles into the antral ovulable stage) and since GnRH receptors, but not as yet GnRH itself, have been shown to be present in ovaries, it stood to reason that GnRH (or an analog thereof), should it be present or normally acting in ovaries, would be an anti-FSH agent and, hence, part of the overall machinery responsible for deciding how many follicles reach the ovulable stage at the end of each estrous cycle. To date, almost all of the signals identified to affect, under physiological conditions, the recruitment of follicles, *i.e.* number of follicles reaching the ovulable stage, are of a stimulatory nature. Of these, FSH and estradiol figure prominently, with LH and PRL playing more or less important roles depending on the species involved. Several attempts have been made to identify atretic signals regulating follicular maturation. Androgens have been postulated to be one such atretic agent

(16–19). This implication stems from correlative studies where atresia correlated with elevated levels of androgenic steroids (16), from studies where androgen injection resulted in inhibition of follicular maturation (17), and from studies where antisera to androgens inhibited the inhibitory effect of low concentrations of hCG on ovarian growth, assumed to be due to androgen formation (18, 19). Interestingly, androgens are simultaneously required for follicular maturation, for they serve as precursors for the stimulatory agent estradiol and, as such, act synergistically with cAMP, the second messenger for FSH action in the granulosa cell, to promote granulosa cell differentiation (20–22). The present report provides strong evidence in support of the concept that ovarian GnRH (or a GnRH-like material) is also an atretic signal, since mere interference with its action by administration of a competitive antagonist resulted in an increased number of ovulable follicles.

It should be pointed out, however, that although the experiments presented here are highly suggestive and indicative of GnRH involvement as an atretic signal in follicular maturation, a second independent assessment will be required to substantiate this hypothesis. Such an assessment may be either interference with the action of endogenous GnRH via immunoneutralization and/or direct identification of ovarian GnRH or a GnRH-like material and the study of its tissue levels, showing correlative changes in endogenous ovarian levels and in number of ovulable follicles.

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